
Live-cell time-lapse imaging and single-cell tracking of in vitro cultured neural stem cells - Tools for analyzing dynamics of cell cycle, migration, and lineage selection.

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Public Summary:

Neural stem cell (NSC) cultures have been considered technically challenging for time-lapse analysis due to high motility, photosensitivity, and growth at confluent densities. We have tested feasibility of long-term live-cell time-lapse analysis for NSC migration and differentiation studies. Here, we describe a method to study the dynamics of cell cycle, migration, and lineage selection in cultured multipotent mouse or human NSCs using single-cell tracking during a long-term, 7-14day live-cell time-lapse analysis. We used in-house made PDMS inserts with five microwells on a glass coverslip petri-dish to constrain NSC into the area of acquisition during long-term live-cell imaging. In parallel, we have defined image acquisition settings for single-cell tracking of cell cycle dynamics using Fucci-reporter mouse NSC for 7days as well as lineage selection and migration using human NSC for 14days. Overall, we show that adjustments of live-cell analysis settings can extend the time period of single-cell tracking in mouse or human NSC from 24-72h up to 7-14days and potentially longer. However, we emphasize that experimental use of repeated fluorescence imaging will require careful consideration of controls during acquisition and analysis.

Scientific Abstract:

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